

Mitochondrial Mix

Combined Approach to Visualizing Oxidative Stress in Real Time

Oxidative stress resulting from mitochondrial dysfunction may play a role in toxicity caused by many different environmental contaminants, including aromatic hydrocarbons and metal ions, but it has been difficult to evaluate the role of mitochondria in oxidative stress using currently available methods. A new study combining new and established imaging techniques to document mitochondrial dysfunction now indicates this integrated approach to real-time imaging in live cells can be useful for studying the role of oxidative stress in toxicologic responses [*EHP* 118(7):902–908; Cheng et al.].

Real-time imaging in live cells to detect products of oxidative stress known as reactive oxygen species (ROS) offers superior temporal and spatial resolution compared with traditional methods such as detecting oxidized lipids, proteins, or DNA. But the accuracy and utility of the fluorescent indicator H2DCF-DA, a reagent commonly used for directly detecting ROS in living cells, is limited.

The authors conducted a set of experiments in which they exposed cultured human skin carcinoma cells to zinc (Zn^{2+}), a ubiquitous contaminant known to induce oxidative stress. Three different fluorescent imaging techniques were used to study effects of Zn^{2+} on mitochondria. The first used the fluorophore PG1 to measure production of the ROS hydrogen peroxide. The scientists found that hydrogen peroxide increased within the cells upon Zn^{2+} exposure and that its production was inhibited with the addition of the mitochondrial

inhibitor CCCP, implicating mitochondria as the source of the Zn^{2+} -induced hydrogen peroxide.

A second experiment used the fluorescent indicator JC-1 to measure changes in mitochondrial membrane potential (the difference in electrical potential between the inside and outside of the mitochondrial membrane) following exposure to Zn^{2+} . When Zn^{2+} was administered, loss of JC-1 fluorescence emission indicated a loss of membrane potential consistent with impaired mitochondrial function.

A third experiment used the genetically encoded fluorescent sensor MTroGFP1 to measure the redox potential of mitochondria after Zn^{2+} exposure. MTroGFP1 associates with mitochondria in transfected cells, causing the mitochondria to fluoresce. However, the investigators observed a change in the fluorescent signals following exposure to Zn^{2+} consistent with a loss of redox potential. In a fourth experiment on mitochondria isolated from live mouse hearts, the authors demonstrated that administering Zn^{2+} resulted in rapid mitochondrial swelling, another indication of a loss of mitochondrial function.

The study shows the value of combining multiple imaging techniques to constitute an integrated approach that permits real-time monitoring of the mechanisms behind oxidative stress within living cells. The results also add to the evidence that oxidative stress induced by Zn^{2+} originates in mitochondria and sheds light on some of the mechanisms that may be involved. Further study is under way to determine the exact sequence of cellular events by which toxicants induce generation of ROS and mitochondrial dysfunction.

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Endotoxin from Biomass Burning

An Underestimated Health Hazard?

Approximately 3 billion people worldwide burn biomass—wood, charcoal, dried animal dung, and crop residue—to heat their homes and cook their food. Biomass often is burned in small, poorly ventilated areas; the resulting smoke exposure frequently causes respiratory infections, primarily among women and children younger than 5 years, who spend the most time around the home fires. Recent findings suggest airborne endotoxin generated from burning biomass may play an important role in the health effects associated with biomass smoke [*EHP* 118(7):988–991; Semple et al.].

According to the World Health Organization, exposure to smoke from biomass burning is responsible for 1.5 million premature deaths annually. Previous research has focused primarily on the mass of airborne fine particulate matter as being responsible for the morbidity and mortality caused by biomass burning. These particles can penetrate deep into the lungs, causing inflammation and both acute and chronic airway and lung damage.

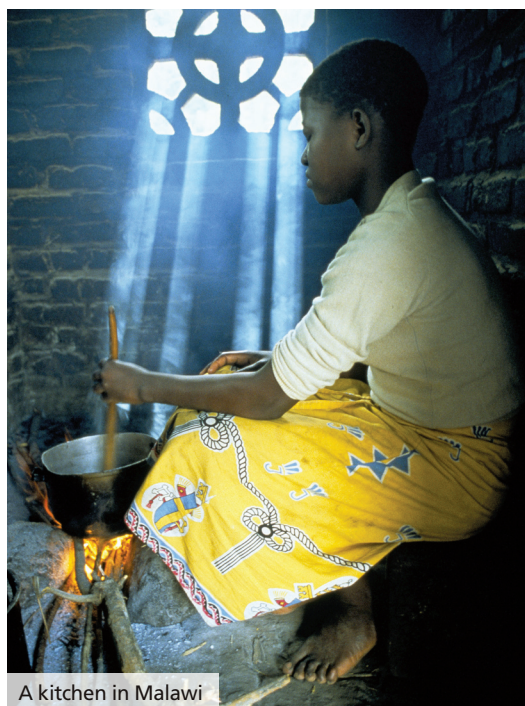
Endotoxins are part of the cell wall of gram-negative bacteria and are found in organic material. These molecules can

cause lung inflammation and have previously been found in tobacco smoke and in homes where there are pets and mold.

To evaluate the presence of airborne endotoxin in homes where biomass is burned, the researchers set up air sampling monitors in 31 homes in Nepal and 38 homes in Malawi. Average levels of inhalable endotoxin measured over 24 hours in Malawian homes were 24 endotoxin units (EU)/m³ for charcoal-burning homes and 40 EU/m³ for wood-burning homes. In Nepal, short-term measurements during cooking indicated average inhalable endotoxin levels of 365 EU/m³ for dung-burning homes and 43 EU/m³ for wood-burning homes. These figures are considerably higher than levels shown to be associated with respiratory ailments during the first two years of life in a separate study [*EHP* 114(4):610–614 (2006)].

The authors acknowledge weaknesses in their study, such as the large time gap between collection of the filters used to trap endotoxins and their analysis, which could have led to high levels of contamination on some of the materials used for collection. Despite the lack of resolution about how biomass smoke contributes to respiratory disease, write the authors, the very fact that it does so makes the use of more efficient stoves and better ventilation in homes where biomass is burned “a matter of urgency.”

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A kitchen in Malawi

Tracking the Deer Tick

Emerging Lyme Disease Threat in Canada

Lyme disease was first diagnosed during an outbreak in the Northeast and upper Midwest of the United States in the late 1970s. Since that time, the disease has become well established in the northeastern United States. A new study now indicates Lyme disease is continuing to spread north into Canada and, because of a convergence of environmental factors, is poised to emerge as a potential public health threat in southern Quebec [EHP 118(7):909–914; Ogden et al.].

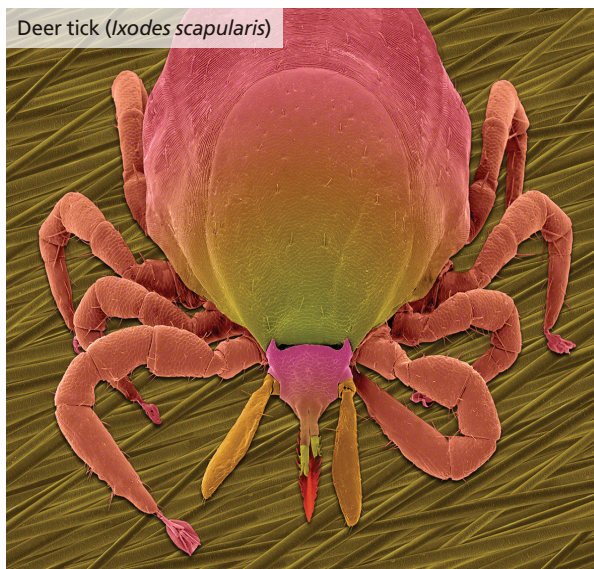
Caused by the bacterium *Borrelia burgdorferi*, Lyme disease is spread through the bite of the deer tick (*Ixodes scapularis*). Symptoms can include skin rash, joint pain, fatigue, and more serious neurologic disorders if the disease is left untreated. Until recently, cooler climate patterns in Canada did not favor the infiltration of *I. scapularis* and consequent spread of Lyme disease, but a warmer climate in southern Quebec may be easing the way for “adventitious” ticks—nonnative ticks introduced most likely by migratory birds—to become established.

In the current study, researchers analyzed data for *I. scapularis* presence and *B. burgdorferi* infection based on passive surveillance (that is, ticks collected voluntarily by medical and veterinary clinics in Quebec were submitted to the provincial public health laboratory) and active surveillance (the research team’s own field analysis of 71 woodland

sites in three regions of southern Quebec) to identify areas where Lyme disease is emerging.

I. scapularis have been collected through passive surveillance in Quebec since 1990, but the investigators observed that between 2004 and 2008 the number of ticks collected increased exponentially to more than 1,700 per year. Given that no marked increase occurred in the number of participating clinics during this time, the increase suggests that in addition to the presence of adventitious ticks, breeding populations of *I. scapularis* have now become established in the region. The authors observed a *B. burgdorferi* infection rate of 13.2% in ticks collected through passive surveillance but a lower prevalence of infection in ticks collected at the active surveillance sites (7.7% overall), implying the *I. scapularis* populations that are becoming established are initially free of *B. burgdorferi*. Ticks that did carry *B. burgdorferi* carried strains that were mostly identical to those seen in the northeastern United States.

The authors postulate that warming climate conditions, a growing tick population, and infected ticks hitchhiking from the United States have set up a favorable scenario for increasing the threat of Lyme disease in southern Quebec. They write that increased surveillance in Quebec and the rest of southeastern Canada would help track the progression of risk areas and protect public health.



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From Roadways to Wheeze

Child Asthma Associated with Traffic Exposures at Home and at School

Approximately 6.2 million children in the United States are affected by asthma, a chronic respiratory disease that is becoming increasingly common in developed countries. Air pollution has been identified as one potential cause for the increase, with proximity of children’s homes to heavy vehicular traffic being a particular investigative focus. However, research results have not been definitive. A unique prospective study has explored the role of traffic-related pollution in causing asthma by estimating children’s exposure both at home and at school against the backdrop of regional ambient air pollution [EHP 118(7):1021–1026; McConnell et al.]. The results associate both school and home exposures with new-onset asthma in young children.

The prospective study included 2,497 children who were asthma-free upon enrollment in the Children’s Health Study during the 2002–2003 school year, when they were in kindergarten or first grade. The cohort represented 13 Southern California communities and 45 schools, and the children were followed for 3 years. Parents completed baseline and yearly surveys providing information on demographic characteristics, respiratory illnesses, and risk factors for asthma. Children with physician-diagnosed asthma, symptoms suggesting undiagnosed asthma, or incomplete health symptom data were excluded from the study.

Temperature, relative humidity, and ambient air levels of ozone, nitrogen dioxide, and particulate matter were measured continuously

at a central monitoring station in each community. Local traffic-related pollutants were estimated for each child’s home and school using a model that incorporated roadway proximity, local traffic density, vehicle emission rates, and meteorologic variables.

During the study, 120 children developed asthma. Development of asthma was independently associated with traffic-related pollution at school and at home; the authors observed a statistically significant association with nitrogen dioxide and nonsignificant associations with fine and ultrafine particulate matter. However, an overall measure of traffic-related pollution (from both freeway and nonfreeway sources) and estimated exposures to individual pollutants were all associated with a significantly increased risk of asthma regardless of whether the exposures occurred at school or at home.

Estimated effects of exposures at school and home were comparable, despite the fact that children spent less time at school than at home. The authors offer possible explanations for this finding: panting during playtime and physical education can increase the dose of pollutants delivered to the lungs, plus children typically arrive at school during morning rush hour, when pollutant levels may be particularly high.

This study strengthens the evidence that nearby vehicular traffic contributes to asthma development and highlights a critical public health concern given that large populations of children are exposed to traffic-related pollutants at school. Controlling vehicular emissions and planning transportation and urban development to limit exposure to traffic-related pollutants could significantly benefit children’s health.

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